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	L #	Hits	Search Text	DBs	Time Stamp
1	L1	354	(alpha adj amylase$1) same bacillus same (mutant$1 or variant$1)	USPAT; US-PGPUB	2003/06/24 09:22
2	L2	5575	(mutant$1 or variant$1) same (stability or thermostabilty or calcium adj depend$8)	USPAT; US-PGPUB	2003/06/24 09:29
3	L3	198	1 and 2	USPAT; US-PGPUB	2003/06/24 09:29

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  * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 13:51:52 ON 24 JUN 2003

=> fil .bec
COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       0.21       0.21

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
       ESBIOBASE, BIOTECHNO, WPIDS' ENTERED AT 13:52:13 ON 24 JUN 2003
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11 FILES IN THE FILE LIST

=> s alpha amylase#
FILE 'MEDLINE'
        447353 ALPHA
         19952 AMYLASE#
L1        4399 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'SCISEARCH'
        630747 ALPHA
         15981 AMYLASE#
L2        7110 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'LIFESCI'
        145006 "ALPHA"
          4245 AMYLASE#
L3        2543 ALPHA AMYLASE#
                 ("ALPHA"(W)AMYLASE#)

FILE 'BIOTECHDS'
         23121 ALPHA
          4710 AMYLASE#
L4        3163 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'BIOSIS'
        587478 ALPHA
         26799 AMYLASE#
L5        9525 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'EMBASE'
        499172 "ALPHA"
         14617 AMYLASE#
L6        3240 ALPHA AMYLASE#
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FILE 'HCAPLUS'
       1408155 ALPHA
         42248 AMYLASE#
L7       17466 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'NTIS'
         28393 ALPHA
           163 AMYLASE#
L8            60 ALPHA AMYLASE#
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FILE 'ESBIOBASE'
        171445 ALPHA
          3652 AMYLASE#
L9        1737 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'BIOTECHNO'
        177086 ALPHA
          4026 AMYLASE#
L10       2049 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'WPIDS'
        162271 ALPHA
          5020 AMYLASE#
L11       2076 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

TOTAL FOR ALL FILES
L12      53368 ALPHA AMYLASE#

=> s l12(3a)(muta? or variant#)
FILE 'MEDLINE'
        409187 MUTA?
         89068 VARIANT#
L13         57 L1 (3A)(MUTA? OR VARIANT#)

FILE 'SCISEARCH'
        386646 MUTA?
         93552 VARIANT#
L14         72 L2 (3A)(MUTA? OR VARIANT#)

FILE 'LIFESCI'
        185562 MUTA?
         30766 VARIANT#
L15         54 L3 (3A)(MUTA? OR VARIANT#)

FILE 'BIOTECHDS'
         34388 MUTA?
         10241 VARIANT#
L16        116 L4 (3A)(MUTA? OR VARIANT#)

FILE 'BIOSIS'
        456180 MUTA?
         93859 VARIANT#
L17        157 L5 (3A)(MUTA? OR VARIANT#)

FILE 'EMBASE'
        332869 MUTA?
         77197 VARIANT#
L18         50 L6 (3A)(MUTA? OR VARIANT#)

FILE 'HCAPLUS'
        418917 MUTA?
         86673 VARIANT#
L19        265 L7 (3A)(MUTA? OR VARIANT#)

FILE 'NTIS'
          9391 MUTA?
          4387 VARIANT#
L20          0 L8 (3A)(MUTA? OR VARIANT#)

FILE 'ESBIOBASE'
        189272 MUTA?
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         32332 VARIANT#
L21         30 L9 (3A)(MUTA? OR VARIANT#)

FILE 'BIOTECHNO'
        226128 MUTA?
         38635 VARIANT#
L22         42 L10(3A)(MUTA? OR VARIANT#)

FILE 'WPIDS'
         21493 MUTA?
         20668 VARIANT#
L23         58 L11(3A)(MUTA? OR VARIANT#)

TOTAL FOR ALL FILES
L24        901 L12(3A)(MUTA? OR VARIANT#)

=> s l12(3a)(Bacillus or termamyl)
FILE 'MEDLINE'
         43147 BACILLUS
             8 TERMAMYL
L25        415 L1 (3A)(BACILLUS OR TERMAMYL)

FILE 'SCISEARCH'
         42526 BACILLUS
            42 TERMAMYL
L26        623 L2 (3A)(BACILLUS OR TERMAMYL)

FILE 'LIFESCI'
         23044 BACILLUS
            12 TERMAMYL
L27        426 L3 (3A)(BACILLUS OR TERMAMYL)

FILE 'BIOTECHDS'
         15368 BACILLUS
            52 TERMAMYL
L28        907 L4 (3A)(BACILLUS OR TERMAMYL)

FILE 'BIOSIS'
         62404 BACILLUS
            63 TERMAMYL
L29        926 L5 (3A)(BACILLUS OR TERMAMYL)

FILE 'EMBASE'
         31699 BACILLUS
            24 TERMAMYL
L30        409 L6 (3A)(BACILLUS OR TERMAMYL)

FILE 'HCAPLUS'
         74546 BACILLUS
           318 TERMAMYL
L31       1972 L7 (3A)(BACILLUS OR TERMAMYL)

FILE 'NTIS'
          1613 BACILLUS
             0 TERMAMYL
L32          4 L8 (3A)(BACILLUS OR TERMAMYL)

FILE 'ESBIOBASE'
         12152 BACILLUS
            15 TERMAMYL
L33        199 L9 (3A)(BACILLUS OR TERMAMYL)

FILE 'BIOTECHNO'
         19004 BACILLUS
```

```
            17 TERMAMYL
L34        371 L10(3A)(BACILLUS OR TERMAMYL)

FILE 'WPIDS'
         10642 BACILLUS
            34 TERMAMYL
L35        178 L11(3A)(BACILLUS OR TERMAMYL)

TOTAL FOR ALL FILES
L36       6430 L12(3A)(BACILLUS OR TERMAMYL)

=> s l24 and l36
FILE 'MEDLINE'
L37         20 L13 AND L25

FILE 'SCISEARCH'
L38         22 L14 AND L26

FILE 'LIFESCI'
L39         17 L15 AND L27

FILE 'BIOTECHDS'
L40         57 L16 AND L28

FILE 'BIOSIS'
L41         45 L17 AND L29

FILE 'EMBASE'
L42         17 L18 AND L30

FILE 'HCAPLUS'
L43        120 L19 AND L31

FILE 'NTIS'
L44          0 L20 AND L32

FILE 'ESBIOBASE'
L45          5 L21 AND L33

FILE 'BIOTECHNO'
L46         10 L22 AND L34

FILE 'WPIDS'
L47         29 L23 AND L35

TOTAL FOR ALL FILES
L48        342 L24 AND L36

=> s l48 not 1997-1999/py
FILE 'MEDLINE'
       1333854 1997-1999/PY
L49         18 L37 NOT 1997-1999/PY

FILE 'SCISEARCH'
       2862496 1997-1999/PY
L50         20 L38 NOT 1997-1999/PY

FILE 'LIFESCI'
        337241 1997-1999/PY
L51         15 L39 NOT 1997-1999/PY

FILE 'BIOTECHDS'
         41041 1997-1999/PY
L52         44 L40 NOT 1997-1999/PY
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FILE 'BIOSIS'
       1680802 1997-1999/PY
L53          41 L41 NOT 1997-1999/PY

FILE 'EMBASE'
       1252969 1997-1999/PY
L54          15 L42 NOT 1997-1999/PY

FILE 'HCAPLUS'
       2535600 1997-1999/PY
L55          86 L43 NOT 1997-1999/PY

FILE 'NTIS'
         85443 1997-1999/PY
L56           0 L44 NOT 1997-1999/PY

FILE 'ESBIOBASE'
        831730 1997-1999/PY
L57           3 L45 NOT 1997-1999/PY

FILE 'BIOTECHNO'
        338670 1997-1999/PY
L58           9 L46 NOT 1997-1999/PY

FILE 'WPIDS'
       2351668 1997-1999/PY
L59          12 L47 NOT 1997-1999/PY

TOTAL FOR ALL FILES
L60         263 L48 NOT 1997-1999/PY

=> s l60 not 2000-2003/py
FILE 'MEDLINE'
       1753010 2000-2003/PY
L61          17 L49 NOT 2000-2003/PY

FILE 'SCISEARCH'
       3309811 2000-2003/PY
L62          19 L50 NOT 2000-2003/PY

FILE 'LIFESCI'
        336333 2000-2003/PY
L63          14 L51 NOT 2000-2003/PY

FILE 'BIOTECHDS'
         61757 2000-2003/PY
L64          34 L52 NOT 2000-2003/PY

FILE 'BIOSIS'
       1803382 2000-2003/PY
L65          30 L53 NOT 2000-2003/PY

FILE 'EMBASE'
       1500007 2000-2003/PY
L66          15 L54 NOT 2000-2003/PY

FILE 'HCAPLUS'
       3330626 2000-2003/PY
L67          64 L55 NOT 2000-2003/PY

FILE 'NTIS'
         55117 2000-2003/PY
L68           0 L56 NOT 2000-2003/PY
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FILE 'ESBIOBASE'
967524 2000-2003/PY
L69 3 L57 NOT 2000-2003/PY

FILE 'BIOTECHNO'
395493 2000-2003/PY
L70 9 L58 NOT 2000-2003/PY

FILE 'WPIDS'
2981530 2000-2003/PY
L71 1 L59 NOT 2000-2003/PY

TOTAL FOR ALL FILES
L72 206 L60 NOT 2000-2003/PY

=> dup rem l72
PROCESSING COMPLETED FOR L72
L73 108 DUP REM L72 (98 DUPLICATES REMOVED)

=> d tot

L73 ANSWER 1 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Laundry detergent containing a **mutant alpha-amylase**;
Bacillus licheniformis recombinant enzyme production by enzyme engineering, for application as a laundry surfactant
AU Barnett C C; Boyer S G; Mitchinson C; Power S D
AN 1997-00549 BIOTECHDS
PI WO 9630481 3 Oct 1996

L73 ANSWER 2 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI New **alpha-amylase variants**;
mutant enzyme construction for improved calcium dependency, substrate binding, cleavage, pH dependent activity and thermostability; application in e.g. surfactant composition
AU Svendsen A; Bisgard-Frantzen H; Borchert T V
AN 1996-12567 BIOTECHDS
PI WO 9623874 8 Aug 1996

L73 ANSWER 3 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Improved bleach-containing cleaning composition;
granular bleach-containing granular surfactant containing **Bacillus** licheniformis **mutant alpha-amylase** and **Bacillus** amyloliquefaciens **mutant** protease
AU Barnett C C; Mitchinson C; Power S D
AN 1996-06019 BIOTECHDS
PI WO 9605295 22 Feb 1996

L73 ANSWER 4 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Acid-stable and thermostable alpha-amylase gene;
characterization from Bacillus licheniformis; enzyme engineering
AN 1997-01693 BIOTECHDS
PI JP 08289788 5 Nov 1996

L73 ANSWER 5 OF 108 HCAPLUS COPYRIGHT 2003 ACS
TI Purification and characterization of a truncated **Bacillus** subtilis .**alpha**.-**amylase** produced by Escherichia coli
SO Applied Microbiology and Biotechnology (1996), 44(6), 746-52
CODEN: AMBIDG; ISSN: 0175-7598
AU Marco, J. L.; Bataus, L. A.; Valencia. F. F.; Ulhoa, C. J.; Astolfi-Filho, S.; Felix, C. R.
AN 1996:206488 HCAPLUS

DN 124:336337

L73 ANSWER 6 OF 108 HCAPLUS COPYRIGHT 2003 ACS
TI Structure-function studies of two polysaccharide-degrading enzymes: **Bacillus** stearothermophilus .**alpha**.-**amylase** and Trichoderma reesei cellobiohydrolase II
SO VTT Publications (1996), 277, 143pp
CODEN: VTTPEY; ISSN: 1235-0621
AU Koivula, Anu
AN 1996:749573 HCAPLUS
DN 126:28530

L73 ANSWER 7 OF 108 HCAPLUS COPYRIGHT 2003 ACS
TI Instability of .alpha.-amylase production and morphological variation in continuous culture of Bacillus amyloliquefaciens is associated with plasmid loss
SO Process Biochemistry (Oxford) (1996), Volume Date 1997, 32(1), 51-59
CODEN: PBCHE5; ISSN: 1359-5113
AU Hillier, P.; Wase, D. A. J.; Emery, A. N.; Solomons, G. L.
AN 1996:695106 HCAPLUS
DN 125:326485

L73 ANSWER 8 OF 108 HCAPLUS COPYRIGHT 2003 ACS
TI Hyperthermostable mutants of Bacillus licheniformis: thermodynamic studies and structural interpretation
SO Perspectives on Protein Engineering '96, [International Conference], 5th, Montpellier, Fr., 1996 (1996), Paper No. 7, 9 pp.. Editor(s): Geisow, Michael J. Publisher: BIODIGM, Bingham, UK.
CODEN: 64HIAR
AU Declerck, Nathalie; Gaillardin, Claude; Machius, Mischa; Wiegand, Georg; Huber, Robert
AN 1997:287296 HCAPLUS
DN 126:314064

L73 ANSWER 9 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI **Mutant** B. licheniformis **alpha-amylase** enzymes;
Bacillus licheniformis mutant thermostable enzyme production; application in starch degradation, textile or paper desizing, brewing industry and as household surfactant
AU van der Laan J M; Aehle W
AN 1996-03039 BIOTECHDS
PI WO 9535382 28 Dec 1995

L73 ANSWER 10 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI New **alpha-amylase variants**;
Bacillus liquefaciens **alpha-amylase** enzyme engineering for improved thermostability, pH stability, etc.; application in surfactant composition to improve washing performance
AU Bisgard-Frantzen H; Borchert T V; Svendsen A; Thellersen M; van der Zee P
AN 1995-07973 BIOTECHDS
PI WO 9510603 20 Apr 1995

L73 ANSWER 11 OF 108 MEDLINE DUPLICATE 1
TI Hyperthermostable **mutants** of **Bacillus** licheniformis **alpha-amylase**: multiple amino acid replacements and molecular modelling.
SO PROTEIN ENGINEERING, (1995 Oct) 8 (10) 1029-37.
Journal code: 8801484. ISSN: 0269-2139.
AU Declerck N; Joyet P; Trosset J Y; Garnier J; Gaillardin C
AN 96367070 MEDLINE

L73 ANSWER 12 OF 108 MEDLINE DUPLICATE 2
TI Effects of signal peptide mutations on processing of **Bacillus**

stearothermophilus **alpha-amylase** in Escherichia coli.
SO MICROBIOLOGY, (1995 Mar) 141 (Pt 3) 649-54.
Journal code: 9430468. ISSN: 1350-0872.
AU Suominen I; Meyer P; Tilgmann C; Glumoff T; Glumoff V; Kapyla J; Mantsala P
AN 95227363 MEDLINE

L73 ANSWER 13 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Bacillus licheniformis, Bacillus stearothermophilus and **Bacillus** amyloliquefaciens **alpha-amylase** enzyme engineering by site-directed mutagenesis;
DNA sequence; application in a surfactant or a starch liquefaction composition
AN 1994-13784 BIOTECHDS
PI WO 9418314 18 Aug 1994

L73 ANSWER 14 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Lipase and **alpha-amylase variant** stabilized against peroxidase system;
Humicola lanuginosa or Bacillus licheniformis enzyme stabilization by enzyme engineering for use in a surfactant composition
AN 1994-11299 BIOTECHDS
PI WO 9414951 7 Jul 1994

L73 ANSWER 15 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI **Mutant alpha-amylase** from **Bacillus** sp. use as surfactant, dish washing agent and liquefaction agent;
Bacillus or Aspergillus spp. thermostable enzyme with increased thermostability and activity at low pH produced by enzyme engineering
AN 1994-04189 BIOTECHDS
PI WO 9402597 3 Feb 1994

L73 ANSWER 16 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Saccharomycopsis fibuligera **alpha-amylase** or **Bacillus** macerans cyclomaltodextrin-glucanotransferase mutant preparation;
enzyme engineering for improved production of oligosaccharide
AN 1995-00657 BIOTECHDS
PI JP 06253836 13 Sep 1994

L73 ANSWER 17 OF 108 MEDLINE DUPLICATE 3
TI Site-directed mutagenesis reveals critical importance of the catalytic site in the binding of alpha-amylase by wheat proteinaceous inhibitor.
SO BIOCHEMISTRY, (1994 Jun 28) 33 (25) 7925-30.
Journal code: 0370623. ISSN: 0006-2960.
AU Takase K
AN 94281224 MEDLINE

L73 ANSWER 18 OF 108 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 4
TI C-TERMINAL TRUNCATIONS OF A THERMOSTABLE **BACILLUS** -STEAROTHERMOPHILUS **ALPHA-AMYLASE**
SO PROTEIN ENGINEERING, (OCT 1994) Vol. 7, No. 10, pp. 1255-1259.
ISSN: 0269-2139.
AU VIHINEN M (Reprint); PELTONEN T; IITIA A; SUOMINEN I; MANTSALA P
AN 94:668008 SCISEARCH

L73 ANSWER 19 OF 108 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 5
TI CHANGES IN OPTIMUM PH AND THERMOSTABILITY OF **ALPHA-AMYLASE** FROM **BACILLUS**-LICHENIFORMIS BY SITE-DIRECTED MUTAGENESIS OF HIS-235 AND ASP-328
SO BULLETIN OF THE KOREAN CHEMICAL SOCIETY, (20 OCT 1994) Vol. 15, No. 10, pp. 832-835.
ISSN: 0253-2964.
AU KIM M S (Reprint); LEE S K; JUNG H S; YANG C H

AN 94:725048 SCISEARCH

L73 ANSWER 20 OF 108 HCAPLUS COPYRIGHT 2003 ACS
TI Experimental study on the technological conditions of .**alpha**.-**amylase** fermentation with **Bacillus mutant**
SO Zhejiang Gongxueyuan Xuebao (1994), (2), 56-61
CODEN: ZGXUEM; ISSN: 1000-209X
AU Zheng, Yuguo; Cao, Xiaoru; Lu, Jianwei; Li, Xiaoqin
AN 1994:653778 HCAPLUS
DN 121:253778

L73 ANSWER 21 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI **Mutant Bacillus** licheniformis **alpha-amylase** promoter, vector and DNA sequence;
application in alpha-amylase, cyclomaltodextrin-glucanotransferase, glucosyltransferase and protease production
AN 1993-09548 BIOTECHDS
PI WO 9310249 27 May 1993

L73 ANSWER 22 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI New **mutant Bacillus** licheniformis **alpha-amylase** signal peptide sequence;
to give reduced processing ambiguity during e.g. human recombinant interleukin-3 protein secretion
AN 1994-01346 BIOTECHDS
PI EP 572088 1 Dec 1993

L73 ANSWER 23 OF 108 HCAPLUS COPYRIGHT 2003 ACS
TI Gene expressing in **Bacillus** licheniformis using especially .**alpha**.-**amylase** promoter **variant**
SO PCT Int. Appl., 63 pp.
CODEN: PIXXD2
IN Joergensen, Steen Troels; Joergensen, Per Linaa
AN 1993:553380 HCAPLUS
DN 119:153380

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9310248	A1	19930527	WO 1992-DK337	19921113
W: FI, JP, KR				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE				
JP 07503363	T2	19950413	JP 1993-508898	19921113
EP 672154	A1	19950920	EP 1992-923721	19921113
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE				
FI 9402227	A	19940513	FI 1994-2227	19940513

L73 ANSWER 24 OF 108 Elsevier BIOBASE COPYRIGHT 2003 Elsevier Science B.V.
DUPLICATE
AN 1994016204 ESBIOBASE
TI Crystallization and preliminary X-ray studies of wild type and catalytic-site **mutant alpha-amylase** from **Bacillus** subtilis
AU Mizuno H.; Morimoto Y.; Tsukihara T.; Matsumoto T.; Takase K.
CS H. Mizuno, Japan.
SO Journal of Molecular Biology, (1993), 234/4 (1283-1293)
CODEN: JMOBAK ISSN: 0022-2836
DT Journal; Article
LA English
SL English

L73 ANSWER 25 OF 108 MEDLINE DUPLICATE 7
TI Crystallization and preliminary X-ray studies of wild type and catalytic-site **mutant alpha-amylase** from **Bacillus** subtilis.
SO JOURNAL OF MOLECULAR BIOLOGY, (1993 Dec 20) 234 (4) 1282-3.

Journal code: 2985088R. ISSN: 0022-2836.
AU Mizuno H; Morimoto Y; Tsukihara T; Matsumoto T; Takase K
AN 94087744 MEDLINE

L73 ANSWER 26 OF 108 MEDLINE DUPLICATE 8
TI Effect of mutation of an amino acid residue near the catalytic site on the activity of **Bacillus** stearothermophilus **alpha-amylase**.
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (1993 Feb 1) 211 (3) 899-902.
Journal code: 0107600. ISSN: 0014-2956.
AU Takase K
AN 93170327 MEDLINE

L73 ANSWER 27 OF 108 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. DUPLICATE 9
TI Transformation of **Bacillus** subtilis in **alpha-amylase** production by **mutant** DNA.
SO Journal of the Chinese Agricultural Chemical Society, (1993) Vol. 31, No. 4, pp. 454-465.
ISSN: 0578-1736.
AU Wang, Mei-Jen; Chou, Cheng-Chun; Yu, Roch-Chui
AN 1994:269713 BIOSIS

L73 ANSWER 28 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Purification and characterization of a thermostable **alpha-amylase** from **Bacillus** licheniformis;
enzyme isolation and properties
SO J.Biotechnol.; (1993) 28, 2-3, 277-89
CODEN: JBITD4
AU Ivanova V N; Dobreva E P; Emanuilova E I
AN 1993-07233 BIOTECHDS

L73 ANSWER 29 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Stability of industrial enzymes;
enzyme stabilization by chemical modification or enzyme engineering (conference paper)
SO Stud.Org.Chem.; (1993) 47, 111-31
CODEN: 9999T
AU Misset O
AN 1994-05917 BIOTECHDS

L73 ANSWER 30 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI New thermostable forms of **Bacillus** licheniformis **alpha-amylase**;
enzyme engineering by specific amino acid substitutions at positions 133 and or 209, for simultaneous gelation and liquefaction of starch, e.g. in brewing
AN 1993-03609 BIOTECHDS
PI FR 2676456 20 Nov 1992

L73 ANSWER 31 OF 108 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 11
TI Thermostable **variants** of **Bacillus** licheniformis **alpha-amylase**, manufacture with transgenic cells, and their use
SO Fr. Demande, 18 pp.
CODEN: FRXXBL
IN Declerck, Nathalie; Joyet, Philippe; Gaillardin, Claude
AN 1993:55127 HCAPLUS
DN 118:55127

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI FR 2665178	A1	19920131	FR 1990-9679	19900730

L73 ANSWER 32 OF 108 MEDLINE DUPLICATE 12

TI Hyperthermostable variants of a highly thermostable alpha-amylase.
SO BIO/TECHNOLOGY, (1992 Dec) 10 (12) 1579-83.
Journal code: 8309273. ISSN: 0733-222X.
AU Joyet P; Declerck N; Gaillardin C
AN 93168398 MEDLINE

L73 ANSWER 33 OF 108 MEDLINE DUPLICATE 13
TI Interaction of catalytic-site **mutants** of **Bacillus** subtilis **alpha-amylase** with substrates and acarbose.
SO BIOCHIMICA ET BIOPHYSICA ACTA, (1992 Aug 21) 1122 (3) 278-82.
Journal code: 0217513. ISSN: 0006-3002.
AU Takase K
AN 92369111 MEDLINE

L73 ANSWER 34 OF 108 MEDLINE DUPLICATE 14
TI Functional relationships between cyclodextrin glucanotransferase from an alkalophilic **Bacillus** and **alpha-amylases**. Site-directed **mutagenesis** of the conserved two Asp and one Glu residues.
SO FEBS LETTERS, (1992 Jan 13) 296 (1) 37-40.
Journal code: 0155157. ISSN: 0014-5793.
AU Nakamura A; Haga K; Ogawa S; Kuwano K; Kimura K; Yamane K.
AN 92111781 MEDLINE

L73 ANSWER 35 OF 108 HCAPLUS COPYRIGHT 2003 ACS
TI Comparison of microbial amylase production by various mutants derived from Bacillus amyloliquefaciens
SO Gongye Weishengwu (1992), 22(2), 26-9
CODEN: GOWEEK; ISSN: 1001-6678
AU Hu, Xuezhi; Hou, Qinfang; Ling, Chen
AN 1992:549367 HCAPLUS
DN 117:149367

L73 ANSWER 36 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI A mutant enzyme with reduced stability; **Bacillus** amyloliquefaciens **alpha-amylase** **mutant** expression in e.g. Escherichia coli, Bacillus, Aspergillus spp.; bread improver with reduced thermostability during baking; DNA sequence
AN 1991-04156 BIOTECHDS
PI EP 409299 23 Jan 1991

L73 ANSWER 37 OF 108 LIFESCI COPYRIGHT 2003 CSA DUPLICATE 15
TI Random point mutation analysis of the signal peptide cleavage area of **Bacillus** stearothermophilus **alpha** -**amylase**.
SO AGRIC. BIOL. CHEM., (1991) vol. 55, no. 11, pp. 2875-2876.
AU Yamaguchi, K.; Ueda, M.; Kawanishi, G.; Udaka, S.
AN 93:1248 LIFESCI

L73 ANSWER 38 OF 108 HCAPLUS COPYRIGHT 2003 ACS
TI Isolation of .alpha.-amylase hyperproducing strain HG4 from Bacillus sp. and some properties of the enzyme
SO Sanop Misaengmul Hakhoechi (1991), 19(5), 464-9
CODEN: SMHAEH; ISSN: 0257-2389
AU Kim, Moo Sung; O, Pyong Su
AN 1993:537438 HCAPLUS
DN 119:137438

L73 ANSWER 39 OF 108 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
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L73 ANSWER 40 OF 108 LIFESCI COPYRIGHT 2003 CSA
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L73 ANSWER 41 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
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L73 ANSWER 42 OF 108 SCISEARCH COPYRIGHT 2003 THOMSON ISI
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L73 ANSWER 43 OF 108 HCAPLUS COPYRIGHT 2003 ACS
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L73 ANSWER 44 OF 108 HCAPLUS COPYRIGHT 2003 ACS
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CODEN: JKXXAF
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	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 02222687	A2	19900905	JP 1989-43561	19890225

L73 ANSWER 45 OF 108 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
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L73 ANSWER 46 OF 108 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
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L73 ANSWER 48 OF 108 SCISEARCH COPYRIGHT 2003 THOMSON ISI
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Journal code: 8801484. ISSN: 0269-2139.
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L73 ANSWER 50 OF 108 HCAPLUS COPYRIGHT 2003 ACS
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SO Weishengwu Xuebao (1990), 30(1), 83-5
CODEN: WSHPA8; ISSN: 0001-6209
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AN 1990:175379 HCAPLUS
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L73 ANSWER 51 OF 108 HCAPLUS COPYRIGHT 2003 ACS
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CODEN: SPYYDO; ISSN: 0253-990X
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DN 113:38799

L73 ANSWER 52 OF 108 HCAPLUS COPYRIGHT 2003 ACS
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CODEN: EPXXDW
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DN 112:53755

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PI	EP 320685	A1	19890621	EP 1988-119794	19881128
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	R: BE, DE, FR, GB, IT, NL				
	ES 2007758	A6	19890701	ES 1987-3510	19871207
	FI 8705567	A	19890608	FI 1987-5567	19871217
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SO Journal of Fermentation and Bioengineering (1989), 67(3), 176-81
CODEN: JFBIEX; ISSN: 0922-338X
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DN 110:191188

L73 ANSWER 56 OF 108 HCAPLUS COPYRIGHT 2003 ACS
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CODEN: AMBIDG; ISSN: 0175-7598
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AN 1989:210892 HCAPLUS
DN 110:210892

L73 ANSWER 57 OF 108 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 20
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L73 ANSWER 58 OF 108 HCAPLUS COPYRIGHT 2003 ACS
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SO Gekkan Fudo Kemikaru (1989), 5(7), 31-7
CODEN: GFKEEX; ISSN: 0911-2286
AU Yamane, Kunio
AN 1989:547515 HCAPLUS
DN 111:147515

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CODEN: WSWPDI; ISSN: 0253-2654
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AN 1989:495531 HCAPLUS
DN 111:95531

L73 ANSWER 60 OF 108 HCAPLUS COPYRIGHT 2003 ACS

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CODEN: EPXXDW
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DN 110:187342

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI EP 285123	A2	19881005	EP 1988-105163	19880330
EP 285123	A3	19890201		
R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
FI 8801530	A	19881004	FI 1988-1530	19880331
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L73 ANSWER 61 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
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L73 ANSWER 62 OF 108 MEDLINE DUPLICATE 21
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Dvukhkodonnyi mutagenez gena alpha-amilazy Bacillus amyloliquefaciens.
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L73 ANSWER 63 OF 108 MEDLINE DUPLICATE 22
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L73 ANSWER 64 OF 108 SCISEARCH COPYRIGHT 2003 THOMSON ISI
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L73 ANSWER 65 OF 108 SCISEARCH COPYRIGHT 2003 THOMSON ISI
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L73 ANSWER 66 OF 108 HCAPLUS COPYRIGHT 2003 ACS

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SO Shengwu Gongcheng Xuebao (1988), 4(2), 119-26
CODEN: SGXUED; ISSN: 1000-3061
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AN 1988:508870 HCAPLUS
DN 109:108870

L73 ANSWER 67 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
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L73 ANSWER 68 OF 108 HCAPLUS COPYRIGHT 2003 ACS
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L73 ANSWER 69 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
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CODEN: DNAADR
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L73 ANSWER 70 OF 108 SCISEARCH COPYRIGHT 2003 THOMSON ISI
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L73 ANSWER 71 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
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CODEN: PRENE9
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AN 1987-11650 BIOTECHDS

L73 ANSWER 72 OF 108 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
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L73 ANSWER 73 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
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-amylase from **Bacillus** licheniformis;
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CODEN: PRENE9
AU Joyet P; Declerck N; Le Reverend B; Heslot H
AN 1987-11496 BIOTECHDS

L73 ANSWER 74 OF 108 SCISEARCH COPYRIGHT 2003 THOMSON ISI
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AN 87:404556 SCISEARCH

L73 ANSWER 75 OF 108 MEDLINE DUPLICATE 24
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L73 ANSWER 76 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
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AN 1989-05994 BIOTECHDS

L73 ANSWER 77 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
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alpha-amylase;
created by site-directed mutagenesis (conference abstract)
SO Protein Engineering 87; (1987) 21
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AN 1989-05993 BIOTECHDS

L73 ANSWER 78 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
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L73 ANSWER 79 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
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Bacillus acidocaldarius and Bacillus stearothermophilus potential strain improvement
SO Agric.Biol.Chem.; (1986) 50, 10, 2633-35
CODEN: ABCHA6
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L73 ANSWER 80 OF 108 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
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CODEN: SMHAEH.
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L73 ANSWER 81 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
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CODEN: BIOHAO
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L73 ANSWER 82 OF 108 HCAPLUS COPYRIGHT 2003 ACS
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CODEN: WSWPDI; ISSN: 0253-2654
AU He, Jichun; Wang, Fengyun; He, Chunhua
AN 1986:475540 HCAPLUS
DN 105:75540

L73 ANSWER 83 OF 108 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
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L73 ANSWER 84 OF 108 HCAPLUS COPYRIGHT 2003 ACS
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CODEN: BIOHAO; ISSN: 0006-307X
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DN 103:50230

L73 ANSWER 85 OF 108 MEDLINE DUPLICATE 27
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Ochistka i nekotorye svoistva al'fa-amilaz morfologicheskikh variantov Bacillus subtilis.
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Journal code: 0023416. ISSN: 0555-1099.
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L73 ANSWER 86 OF 108 HCAPLUS COPYRIGHT 2003 ACS
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CODEN: 54VYAW
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AN 1986:65774 HCAPLUS
DN 104:65774

L73 ANSWER 87 OF 108 HCAPLUS COPYRIGHT 2003 ACS
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SO Eur. Pat. Appl., 20 pp.
CODEN: EPXXDW
IN Dean, Donald H.; Ellis, Daniel M.
AN 1984:3412 HCAPLUS
DN 100:3412

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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	EP 92235	A3	19840912		
	R: BE, CH, DE, FR, IT, LI, NL, SE				
	US 4465773	A	19840814	US 1982-370433	19820421
	AU 8313221	A1	19831027	AU 1983-13221	19830407
	AU 555540	B2	19861002		
	ZA 8302452	A	19831228	ZA 1983-2452	19830407
	IL 68378	A1	19860228	IL 1983-68378	19830414
	DK 8301733	A	19831022	DK 1983-1733	19830420
	GB 2118968	A1	19831109	GB 1983-10737	19830420
	GB 2118968	B2	19850717		
	JP 58190391	A2	19831107	JP 1983-69280	19830421

L73 ANSWER 88 OF 108 HCAPLUS COPYRIGHT 2003 ACS
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CODEN: EPXXDW
IN Yoneda, Yuko; Young, Frank E.
AN 1983:433890 HCAPLUS
DN 99:33890

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 74553	A2	19830323	EP 1982-107959	19820830
	EP 74553	A3	19850109		
	R: AT, BE, CH, DE, FR, GB, IT, LI, NL, SE				
	IL 66758	A1	19860228	IL 1982-66758	19820909
	DK 8204062	A	19830312	DK 1982-4062	19820910
	NO 8203080	A	19830314	NO 1982-3080	19820910
	ES 515620	A1	19840401	ES 1982-515620	19820910
	JP 58067186	A2	19830421	JP 1982-158725	19820911
	JP 62053150	B4	19871109		
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L73 ANSWER 89 OF 108 MEDLINE DUPLICATE 28
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L73 ANSWER 90 OF 108 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
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L73 ANSWER 91 OF 108 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI DIRECT ACTIVITY STAINS FOR GLYCOSIDASE AND GLUCOSYL TRANSFERASE AFTER ISO ELECTRIC FOCUSING IN HORIZONTAL POLY ACRYLAMIDE GEL LAYERS.
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L73 ANSWER 108 OF 108 HCAPLUS COPYRIGHT 2003 ACS
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=> d ab 9,11,14,17,26,32,35,44,45,47,52,62

L73 ANSWER 9 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AB An amylolytic enzyme (I) derived from **Bacillus** licheniformis **alpha-amylase** (EC-3.2.1.1) (or an enzyme with 70% identity) is new, containing 1 or more amino acid changes at position 104 (Asn to Asp), 128 (Val to Glu), 187 (Ser to Asp) and 188 (Asn to Asp) of the wild-type enzyme. Also claimed are: a nucleic acid encoding (I); a vector for the expression of (I); host cells expressing (I); and, a detergent composition containing (I). (I) preferably has an additional amino acid change, providing the enzyme with increased thermostability, preferably the mutations His 133 to Tyr 133 and Thr 149 to Ile 149. (I) may also have at least 1 amino acid change providing the enzyme with improved oxidation stability, preferably by changing a Met residue to another amino acid, e.g. Met 197. The mutant enzyme has higher activity under optimal and suboptimal conditions (pH less than 6.5 or over 7 and/or Ca2+ concentration under 50 ppm). (I) may be used for starch degradation (syrup, isosyrup or ethanol production), in textile or paper desizing, in the brewing industry and in household surfactant compositions. (32pp)

L73 ANSWER 11 OF 108 MEDLINE DUPLICATE 1
AB We have identified previously two critical positions for the thermostability of the highly thermostable **alpha-amylase** from **Bacillus** licheniformis. We have now introduced all 19 possible amino acid residues to these two positions, His133 and Ala209. The most favourable substitutions were to Ile and Val, respectively, which both increased the half-life of the enzyme at 80 degrees C by a factor of approximately 3. At both positions a stabilizing effect of hydrophobic residues was observed, although only in the case of position 133 could a clear correlation be drawn between the hydrophobicity of the inserted

amino acid and the gain in protein stability. The construction of double mutants showed a cumulative effect of the most favourable and/or deleterious substitutions. Computer modelling was used to generate a 3-D structure of the wild-type protein and to model substitutions at position 209, which lies in the conserved (alpha/beta)8 barrel domain of alpha-amylase; Ala209 would be located at the beginning of the third helix of the barrel, in the bottom of a small cavity facing the fourth helix. The model suggests that replacement by, for example, a valine could fill this cavity and therefore increase intra- and interhelical compactness and hydrophobic interactions.

L73 ANSWER 14 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AB A new lipase (EC-3.1.1.3) or **alpha-amylase** (EC-3.2.1.1) **variant**, stabilized towards inactivation caused by a peroxidase (EC-1.11.1.7) system (a peroxidase, an H2O2 source and an enhancing agent) has at least 1 native Tyr residue deleted or substituted with Phe, Leu, Ile, Val, Gln, Asn, Ser, Thr, Glu or His. The lipase variant is from Humicola lanuginosa (preferred), Humicola brevispora, Humicola brevis var. thermoidea, Humicola insolens, Pseudomonas cepacia, Pseudomonas fragi, Pseudomonas stutzeri, Pseudomonas fluorescens, Fusarium oxysporum, Rhizomucor miehei, Candida antarctica or Candida cylindracea, and has a mutation at position 16, 21, 53, 138, 164, 171, 194, 213, 220 or 261. The **alpha-amylase** **variant** is from **Bacillus** licheniformis (preferred) or Aspergillus sp., and has a mutation at position 10, 14, 31, 46, 56, 59, 62, 77, 98, 150, 158, 175, 193, 195, 198, 203, 219, 262, 273, 290, 302, 348, 358, 363, 367, 394, 396, 402, 439 or 480. The enzymes are useful in surfactant compositions, and have improved compatibility with peroxidase systems used to inhibit dye transfer. (28pp)

L73 ANSWER 17 OF 108 MEDLINE DUPLICATE 3
AB A bacterial **alpha-amylase** from **Bacillus** subtilis was found to be strongly inhibited by wheat alpha-amylase inhibitors 0.53 and 0.19, which had previously been thought specific for animal alpha-amylase. Inhibition and gel filtration studies of site-directed **mutants** of B. subtilis **alpha-amylase** with the inhibitors indicated a direct correlation between the alpha-amylase activity and the inhibitory effect of inhibitor binding. A mutant enzyme His 180-->Asn, which was 20 times less active in terms of kcat than the wild type, was less sensitive to inhibition by similar degrees, while the specificity for 0.53 and 0.19 changed significantly as a result of the mutation. Catalytic-site mutants that were completely devoid of catalytic activity virtually lost the ability to bind inhibitors, even though they retained high affinities for substrates. The results show that the integrity of the catalytic site is crucial for inhibitor binding and, despite the previously observed tight binding, reveal a subtle nature of the interaction between alpha-amylase and the wheat inhibitor, which leads to a proposal of a two-step mechanism for the binding interaction.

L73 ANSWER 26 OF 108 MEDLINE DUPLICATE 8
AB Site-directed **mutagenesis** of a thermostable **alpha-amylase** from **Bacillus** stearothermophilus was performed to assess the role of amino acid residues near the catalytic site in catalysis. Asn329 is presumed to be adjacent to the proposed catalytic residue Asp331. Its mutation to Lys, which is found at the corresponding position in pullulanase, resulted in the loss of 99.7% of the activity, while the mutation to Asp or Val did not drastically reduce the activity. The mutation to Val altered the temperature/activity profile so that the activity was reduced to 25% of wild-type alpha-amylase at 60 degrees C but was over twofold greater at 5 degrees C. This effect could be ascribed to a decrease in the activation enthalpy by 32%. The mutation to Asp or Lys altered the pH/activity profile concomitant with possible changes in the ionization state of the groups introduced. These results show the

feasibility of altering and possibly improving the enzyme activity by mutagenesis of residues near the catalytic groups.

L73 ANSWER 32 OF 108 MEDLINE DUPLICATE 12
AB Genetic screening at temperatures between 70-80 degrees C far exceeds the range of growth of most bacteria, and is not applicable to isolate easily thermostable protein variants. We describe a temperature shift protocol and an in vivo screening method which allowed us to identify a hyperthermostable **variant** of the thermostable **alpha-amylase** from **Bacillus** licheniformis. Our strategy was to select, after hydroxylamine mutagenesis, an intragenic suppressor mutation which overcomes a mutation leading to a thermolabile enzyme. Sequence analysis of the mutated gene revealed only one change in the amino acid sequence, substituting a valine for alanine at position 209. This single amino acid replacement increased the half-life of the protein at 90 degrees C by a factor of two to three relative to the wild-type enzyme. When this substitution was combined with another stabilizing substitution (H133Y) we described previously, the stabilizing effects were additive. The half-life of the new protein was about 12 hours at 90 degrees C, corresponding to a nine to ten-fold increase over the wild-type enzyme and the industrial **Bacillus** licheniformis **alpha-amylase Termamyl**. These mutations are located in a predicted folding domain of the protein which appears crucial in determining thermal stability.

L73 ANSWER 35 OF 108 HCAPLUS COPYRIGHT 2003 ACS
AB Several microorganism **mutants** which produced .**alpha**.-**amylase** were isolated from the parent strain BF7658. The strains produced amylase as well as alk. proteinase. Optimal pH was 6. Ca2+ at >0.6% suppressed enzyme prodn.

L73 ANSWER 44 OF 108 HCAPLUS COPYRIGHT 2003 ACS
AB B. subtilis .alpha.-amylase (I) C-terminal deletion mutants having higher sp. activity than wild type I are given. These I mutants have enzymic activity in broader pH range than the wild type I. The deletion (123-468 bp) is made at 3' end of the codon for Lys480 of I. I gene was cloned from B. subtilis and used to prep. expression plasmid pTC237-76 from which 3 expression plasmids encoding deletion mutants were prepd. Into B. subtilis plasmid pTB53, the wild I gene and mutant genes were subcloned and transformed into B. subtilis. B. subtilis transformants produced I and its deletion mutants by aerobic fermn. The deletion mutants of I had sp. activities of 624, 582, and 609 unit/mg, resp.; the wild type I had sp. activity of 223 unit/mg. Moreover, the deletion mutants of I had optimal pH at 5.5-7.5, as compared to 5.5-6.5 for that of the wild type I.

L73 ANSWER 45 OF 108 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

L73 ANSWER 47 OF 108 MEDLINE DUPLICATE 17
AB The relationship between structure, activity, and stability of the thermostable **Bacillus** stearothermophilus **alpha-amylase** was studied by site-directed mutagenesis of the three most conserved residues. Mutation of His-238 to Asp involved in Ca2+ and substrate binding reduced the specific activity and thermal stability, but did not affect the pH and temperature optima. Replacement of Asp-331 by Glu in the active site caused almost total inactivation. Interestingly, in prolonged incubation this mutant enzyme showed an altered end-product profile by liberating only maltose and maltotriose. Conservative mutation of the conserved Arg-232 by Lys, for which no function has yet been proposed, resulted in lowered specific activity: around 12% of the parental enzyme. This mutant enzyme had a wider pH range but about the same temperature optimum and thermal stability as the wild-type enzyme. Results obtained with different mutants were interpreted by computer aided molecular modeling.

L73 ANSWER 52 OF 108 HCAPLUS COPYRIGHT 2003 ACS
AB The title enzyme (I) is manufd. by culturing Bacillus NCIB 12563 at 50-70.degree., preferably 55-65.degree., and recovering I from the culture medium. Bacillus NCIB 12563, an over-prodn. mutant of parental strain 11886, was shake-cultured in medium contg. corn steep liquor, NH4Cl, sol. starch, and yeast ext. at 56.degree. for 24 h to produce I 917 units/mL compared to 216 for that of the parental strain 11886. I was isolated from the culture medium by fractionation with acetone (4.degree.). Prodn. of I on an industrial scale was given.

L73 ANSWER 62 OF 108 MEDLINE DUPLICATE 21
AB The oligonucleotide encoding Bam HI recognition site having the structure pCGGGATC had been inserted into the recognition sites MspI of the B. amyloliquefaciens alpha-amylase gene, which was cloned in pTG29B plasmid. The alpha-amylase gene had no BamHI sites before mutagenesis. The set of pNSBamHI plasmids with BamHI site at four different positions was obtained. It was shown that all the **mutant alpha-amylases** possess different specific activities. One of the mutant proteins possesses reduced thermostability. The **mutant alpha-amylases** can be used for further experiments on protein-engineering of liquefying-type alpha-amylases.

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